

SEQUENCE LISTING

<110> Chang, Chia-Hwa Liu, Xiaowen Lewicki, John A. Xu, Qiang Osel, Inc.

- <120> Surface Expression of Biologically Active Proteins in Bacteria
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- <150> US 60/443,619
- <151> 2003-01-29
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- Ala Leu Gln Asn Lys Asn Tyr Thr Asn Ala Ala Asn Pro Trp Asn Ile
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- Thr Leu Lys Asp Met Thr Ile Glu Gly Ser Lys Tyr Asp Tyr Ser Pro 85 90 95

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Gly Thr Leu Ile Arg Met Glu Gly Val Tyr Asn Ser Thr Thr Val Asn 425 420 Gly Pro Thr Pro Val Ala Gln Trp Asp Gln Gly Asn Lys Thr Thr Thr 440 Pro Asn Asp Val Trp Tyr Val Arg Tyr Leu Ile Ser Ala Asn Gln Trp 455 Gly Asn Asn Ser Gly Gln Phe Met Gly Lys Asp Gln His Pro Asn Thr Val Val Ala Lys Lys Gly Val Asp Thr Leu Tyr Asn Ser Asn Ala Thr 490 Val Leu Met Ser Lys Asn Gln Gly Ala Asp Lys Tyr Glu Asn Gly Thr Met Pro Thr Glu Val Gln Gln Ala Leu His Leu Asn Ser Phe Leu Asn 520 Asn Phe Asn Phe Trp Arg Pro Gln Arg Met Ala Met Gly Ser Lys Leu 535 Asn Asp Asn Pro Asp Val Lys Ile Asp Asp Phe Asp Lys Tyr His Ala 550 555 Glu Ala Gln Thr Ile Asp Gly Thr Thr Arg Gln Thr Leu Ser Asp Leu 570 Asp Ala Asn Lys Gly Leu Lys Asp Leu Ile Gly Pro Asp Glu Gln Pro Ile Thr Asp Phe Lys Asp Ile Val Lys His Val Thr Trp Tyr Asn Ser Ala Thr Asp Lys Asp Glu Trp Asn Lys Ile Met Ile Gln Pro Thr Asp Ser Lys Asp Pro Ser Ala Arg Val Pro Tyr Pro Glu Pro Gln Asn Pro Thr Gly Asn Leu Lys Thr Thr Asp Gly Phe Ala Trp Ala Lys Val Thr 650 Tyr Ala Asp'Gly Ser Val Asp Phe Val Lys Ile Pro Leu Lys Val Thr 665 Glu Lys Lys Tyr Ser Glu Glu Leu Thr Pro Ser Tyr Pro Gly Val Ser 675 680 Val Glu Gln Gly Lys Ser Asp Ser Val Asp Pro Ser Phe Lys Asp Glu Asn Asp Lys Ala Ala Asp Ala Pro Ala Gly Thr Lys Tyr Thr Ala Gly 710 715 Glu Asn Thr Pro Asp Trp Ile Lys Val Asp Pro Asp Thr Gly Lys Val 725 730 735

Thr Val Ser Pro Thr Asp Asp Thr Ser Val Gly Ser His Asp Ile Ser 745 Val Thr Val Thr Tyr Pro Asp Ser Ser Thr Asp Gln Leu Thr Val Pro 760 Val Thr Val Thr Glu Lys Ser Asn Leu Ala Glu Lys Tyr Pro Val Ser 775 Tyr Asp Lys Leu Asn Val Glu Lys Pro Ser Gly Asp Thr Pro Ala Thr Gly Ala Val Asp Pro Lys Ala Ala Ala Asp Met Pro Glu Gly Ala Ile Thr Gly Tyr Glu Lys Gly Asp Phe Asp Ala Pro Ala Gly Val Thr Ile Asp Val Asn His Asp Thr Gly Lys Val Thr Ala Ser Val Gly Lys Asn Ala Thr Leu Gly Ser Phe Glu Val Pro Val Lys Val Thr Tyr Ser Asp 855 Gly Thr Tyr Ala Glu Val Lys Val Pro Val Ser Ile Thr Gly Asn Lys 870 Val Asp Pro Gly Ser Gly Asp Val Val Tyr Tyr Gly Asp Gln Ser Met 890 Val Val Phe Asn Gly Asn Leu Thr Thr Val His Lys Thr Thr Asp Ser His Glu Leu Ser Ala Lys Asp Ser Ala Phe Gln Thr Ile Thr Tyr Tyr Ser Asp Trp Asn Lys Lys Gly Asn Ile Val Ser Asp Tyr Asn Lys His Val Ile Tyr Lys Leu Ser Ala Asp Gly Thr Lys Tyr Val Asn Glu Ala Asp Ala Thr Asp Ser Phe Asp Ala Ser Ala Ile Ser Phe Asn Trp Gln 965 Lys Gly Tyr Glu Val Asn Thr Gly Val Asp Asn Phe Ser Asn Gly Ser Ala Asp Thr Leu Tyr Gln Leu Glu Lys Gly Ala Val Asn Ser Glu Glu 995 1000 Gln Thr Asp Ala Asn Asp Pro Ser Gly Leu Ala Gly Asn Ser Lys Tyr 1015 Arg Tyr Asp Phe Ser Ile Ser Asp Thr Asn Val Leu Gln Lys Leu Gly 1025 1030 1035 Leu Ser Pro Ala Gly Tyr Asn Ala Trp Ala Asn Val Tyr Tyr Asn Phe 1045 1050

- Leu Gly Ala Thr Gly Lys Ile Asn Ile Pro Val Asn Tyr Gly Ser Glu
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- Ala Glu Asn Gly Met Pro Gly Lys Asp Gly Lys Phe Ala Ala Ser Asn 1105 1110 1115 1120
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- Val Gln Val Thr Phe Lys Thr Gly Ser His Val Ser Thr Ser Gly Ser 1140 1145 1150
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- Thr Asn Glu Asp Lys Asp Gly Asn Ser Gly Tyr Lys Asp Pro Val Thr 1315 1320 1325
- Gly Glu Ile Lys Tyr Asn Thr Asp Trp His Val Ala Ser Asp Leu Asn 1330 1335 1340
- Ala Lys Thr Gly Ser Trp Glu Glu Tyr Thr Ala Pro Ser Val Thr Gly 1345 1350 1355 1360
- Tyr Thr Pro Ser Gln Ala Lys Val Glu Ala Lys Thr Val Thr Ala Glu 1365 1370 1375

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- Pro Val Pro Tyr Lys Pro Gly Lys Asp Gly Val Asn Asp Ala Ile Asn 1395 1400 1405
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- Gln Thr Ile Thr Gln Thr Val His Phe Thr Asn Glu Asp Lys Asp Gly 1425 1430 1435 1440
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- Asp Trp His Val Ala Ser Asp Leu Asn Ala Lys Thr Gly Ser Trp Glu 1460 1465 1470
- Glu Tyr Thr Ala Pro Ser Val Thr Gly Tyr Thr Pro Ser Gln Ala Lys 1475 1480 1485
- Val Glu Ala Lys Thr Val Thr Ala Glu Thr Glu Ala Ala Ser Val Thr 1490 1495 1500
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- Asn Lys Asp Met Tyr Arg Glu Val Thr Arg Thr Ile Asn Val Val Asp 1525 1530 1535
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- Lys Asp Thr Val Pro Asp Pro Ala Asp Gly Ile Lys Asn Lys Asp Asp 1635 1640 1645
- Leu Pro Asp Gly Thr Lys Tyr Thr Trp Lys Glu Val Pro Asp Val Asn 1650 1655 1660
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- Asp Thr Lys Gln Ile Ile Ala Lys Tyr Lys Gly Asn Ile Pro Gln Ala 65 70 75 80
- Ser Asp Gly Ile Ala Asn Lys Asp Gln Ala Thr Lys Glu Gly Asp Lys 85 90 95
- Asp Phe Pro Ser Leu Ala Asp Val Leu Ala Pro Asn Gly Ile Gln Trp
- Lys Lys Asn Phe Glu Pro Asp Leu Ser Lys Pro Gly Leu Thr Ser Gly 115 120 125

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- Thr Lys His Ile Thr Asp Glu Asp Lys Gly Lys Val Ile Asp Asn Val 770 780
- Lys Lys Ser Asn Pro Asp Lys Asp Ile Thr Asp Ala His Val Asp Asp 785 790 795 800
- Asp Gly Thr Phe His Gly Lys Val Asp Gly Gln Asp Val Val Ile Pro 805 810 815
- Gly Thr Glu Thr Val Val Glu Lys Gln Lys Glu Ser Leu Asn Pro Pro 820 825 830
- Thr Asp Lys Val Pro Val Asp Asp Thr Lys His Ile Thr Asp Glu Asp 835 840 845
- Lys Gly Lys Val Ile Asp Asn Val Lys Lys Ser Asn Pro Asp Lys Asp 850 855 860
- Ile Thr Asp Ala His Val Asp Asp Gly Thr Phe His Gly Lys Val 865 870 875 886
- Asp Gly Gln Asp Val Val Ile Pro Gly Ile Glu Thr Val Val Glu Lys
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- Ser Thr Asn Asn Gln Lys Ser Asp Thr Asn Lys Gly Leu Ile Ser Asn 900 905 910
- Asp Asn Ser Glu Lys Asn Ser His Met Ile Asn Ala Asn Val Asn Thr 915 920 925
- Lys Ser Arg Asn Ser Leu Ser Ala Lys Gln Asn Arg Leu Pro Gln Thr 930 935 940
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- Glu Ile Thr Thr Ala Lys Asn Asn Ala Val Ser Thr Asp Thr Asn Met 980 985 990
- Lys Asp Leu Ser Glu Asp Thr Lys Leu Ala Ala Asp Lys Thr Gln Asp 995 1000 1005
- Pro Tyr Leu Asn Ala Asp Leu Asp Lys Lys Gln Ala Tyr Asp Lys Ala 1010 1015 1020
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- Ser Asp Asp Thr Lys Ser Lys Phe Asp Glu Ala Leu Lys Lys Ala Glu 1300 1305 1310
- Glu Val Lys Asn Asn Ser Asn Ala Thr Gln Lys Glu Val Asp Asp Ala 1315 1320 1325
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- Asp Lys Ser Lys Leu Asp Asp Ala Ile Lys Asp Ala Asn Asn Ala Lys 1345 1350 1355 1360
- Gly Thr Asp Lys Tyr Lys Asn Ala Ser Asp Asp Thr Lys Ser Lys Phe 1365 1370 1375
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- Asn Asp Leu Asp Gly Gln Thr Thr Asp Lys Ser Lys Leu Asp Glu Ala 1410 1415 1420
- Ile Thr Asp Ala Asn Asn Thr Lys Leu Thr Asp Lys Tyr Asn Asn Ala 1425 1430 1435 1440
- Ser Asp Asp Thr Lys Ser Lys Phe Asp Glu Ala Leu Lys Lys Ala Glu 1445 1450 1455
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- Thr Asn Asn Leu Lys Gln Ala Gln Asn Asp Leu Asp Gly Gln Thr Thr 1475 1480 1485
- Asp Lys Ser Lys Leu Asp Glu Ala Ile Thr Asp Ala Asn Asn Thr Lys 1490 1495 1500
- Ser Thr Asp Lys Tyr Asn Asn Ala Ser Asp Asp Thr Lys Ser Lys Phe 1505 1510 1515 1520
- Asp Glu Ala Leu Lys Lys Ala Glu Glu Val Lys Asn Asn Ser Asn Ala 1525 1530 1535
- Thr Gln Lys Glu Val Asp Asp Ala Thr Asn Asn Leu Lys Gln Ala Gln 1540 1545 1550
- Asn Asn Leu Asp Gly Gln Thr Thr Asp Lys Ser Lys Leu Asp Glu Ala 1555 1560 1565
- Ile Thr Asp Ala Asn Asn Thr Lys Ser Thr Asp Lys Tyr Lys Asn Ala 1570 1575 1580
- Ser Asp Asp Thr Lys Ser Lys Phe Asp Asp Ala Leu Lys Lys Ala Glu 1585 1590 1595 1600

- Glu Val Lys Asn Asn Ser Asn Ala Thr Gln Lys Glu Val Asp Asp Ala 1605 1610 1615
- Thr Asn Asn Leu Lys Gln Ala Gln Asn Asp Leu Asp Gly Gln Thr Thr 1620 1625 1630
- Asn Lys Asp Thr Leu Asn Asp Ala Ile Lys Asp Ala Asn Asp Ala Lys 1635 1640 1645
- Gly Thr Asp Lys Tyr Lys Asn Ala Ser Asp Asp Thr Lys Ser Lys Leu 1650 1660
- Asp Glu Thr Leu Lys Lys Ala Glu Glu Val Lys Asn Asn Ser Asn Ala 1665 1670 1675 1680
- Thr Gln Lys Glu Val Asp Asp Ala Thr Asn Asn Leu Lys Gln Ala Gln 1685 1690 1695
- Asn Asp Leu Asp Gly Gln Thr Thr Asp Lys Ser Lys Leu Asp Glu Ala 1700 1705 1710
- Ile Lys Ser Ala Asp Asp Thr Lys Ser Thr Asp Lys Tyr Asn Asn Ala 1715 1720 1725
- Ser Asp Asp Thr Lys Ser Lys Phe Asp Glu Ala Leu Lys Lys Ala Glu 1730 1735 1740
- Glu Val Lys Asn Asn Ser Asn Ala Thr Gln Lys Glu Val Asp Asp Ala 1745 1750 1755 1760
- Thr Lys Asn Leu Lys Gln Ala Gln Asn Asp Leu Asp Gly Gln Thr Thr 1765 1770 1775
- Asn Lys Asp Ala Ile Asn Asp Ala Ile Lys Asp Ala Asn Asn Ala Lys
 1780 1785 1790
- Gly Thr Asp Lys Tyr Asn Asn Ala Ser Asp Asp Thr Lys Ser Lys Phe 1795 1800 1805
- Asp Asp Ala Leu Lys Lys Ala Glu Asp Val Lys Asn Asp Ser Asn Ala 1810 1815 1820
- Asn Gln Lys Glu Val Asp Asp Ala Thr Lys Asn Leu Lys Asn Thr Leu 1825 1830 1835 1840
- Asn Asn Leu Lys Gly Gln Pro Ala Lys Lys Ala Asn Leu Ile Ala Ser 1845 1850 1855
- Lys Asp Asn Ala Lys Ile His Lys Gln Thr Leu Leu Pro Gln Thr Gly 1860 1865 1870
- Thr Glu Thr Asn Pro Leu Thr Ala Ile Gly Ile Gly Leu Met Ala Leu 1875 1880 1885
- Gly Ala Gly Ile Phe Ala Lys Lys Lys Arg Lys Asp Asp Glu Ala 1890 1895 1900

- <210> 4
- <211> 199
- <212> PRT
- <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:C370 cell wall
 associated (CWA) region

<400> 4

Lys Lys Ala Glu Glu Val Lys Asn Asn Ser Asn Ala Thr Gln Lys Glu

1 10 15

Val Asp Asp Ala Thr Asn Asn Leu Lys Gln Ala Gln Asn Asp Leu Asp 20 25 30

Gly Gln Thr Thr Asp Lys Ser Lys Leu Asp Glu Ala Ile Lys Ser Ala 35 40 45

Asp Asp Thr Lys Ser Thr Asp Lys Tyr Asn Asn Ala Ser Asp Asp Thr 50 55 60

Lys Ser Lys Phe Asp Glu Ala Leu Lys Lys Ala Glu Glu Val Lys Asn 65 70 75 80

Asn Ser Asn Ala Thr Gln Lys Glu Val Asp Asp Ala Thr Lys Asn Leu 85 90 95

Lys Gln Ala Gln Asn Asp Leu Asp Gly Gln Thr Thr Asn Lys Asp Ala 100 105 110

Ile Asn Asp Ala Ile Lys Asp Ala Asn Asn Ala Lys Gly Thr Asp Lys
115 120 125

Tyr Asn Asn Ala Ser Asp Asp Thr Lys Ser Lys Phe Asp Asp Ala Leu 130 135 140

Lys Lys Ala Glu Asp Val Lys Asn Asp Ser Asn Ala Asn Gln Lys Glu 145 150 155 160

Val Asp Asp Ala Thr Lys Asn Leu Lys Asn Thr Leu Asn Asn Leu Lys 165 170 175

Gly Gln Pro Ala Lys Lys Ala Asn Leu Ile Ala Ser Lys Asp Asn Ala 180 185 190

Lys Ile His Lys Gln Thr Leu 195

- <210> 5
- <211> 95
- <212> PRT
- <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:C370 cell wall associated fragment

- <400> 5
- Gly Gln Thr Thr Asn Lys Asp Ala Ile Asn Asp Ala Ile Lys Asp Ala 1 5 10 15
- Asn Asn Ala Lys Gly Thr Asp Lys Tyr Asn Asn Ala Ser Asp Asp Thr \cdot 20 $\qquad \qquad 25 \qquad \qquad 30$
- Lys Ser Lys Phe Asp Asp Ala Leu Lys Lys Ala Glu Asp Val Lys Asn 35 40 45
- Asp Ser Asn Ala Asn Gln Lys Glu Val Asp Asp Ala Thr Lys Asn Leu 50 55 60
- Lys Asn Thr Leu Asn Asn Leu Lys Gly Gln Pro Ala Lys Lys Ala Asn 65 70 75 80
- Leu Ile Ala Ser Lys Asp Asn Ala Lys Ile His Lys Gln Thr Leu 85 90 95
- <210> 6
- <211> 202
- <212> PRT
- <213> Artificial Sequence
- <220>
- <400> 6
- Val Thr Arg Thr Ile Asn Val Val Asp Pro Ile Thr Gly Lys Ile Ser 1 5 10 15
- Thr Ser Val Gln Thr Ala Lys Phe Thr Arg Glu Asp Lys Asn Ser Asn 20 25 30
- Ala Gly Tyr Thr Asp Pro Val Thr Gly Lys Thr Thr Met Asn Pro Trp 35 40 45
- Thr Pro Ala Lys Gln Gly Leu Arg Ala Val Asn Val Glu Gln Ile Lys
 50 55 60
- Gly Tyr Val Ala Lys Val Asp Gly Asn Val Asp Ala Val Val Val Thr
 65 70 75 80
- Pro Asp Ser Ala Asn Met Val Val Thr Ile Thr Tyr Gln Ala Asn Lys 85 90 95
- Pro Glu Gly Gln Asn Ile Thr Val Lys Lys Asp Thr Val Pro Asp Pro 100 105 110
- Ala Asp Gly Ile Lys Asn Lys Asp Leu Pro Asp Gly Thr Lys Tyr 115 120 125
- Thr Trp Lys Glu Val Pro Asp Val Asn Ser Val Gly Glu Lys Thr Gly 130 135 140
- Ile Val Thr Val Thr Phe Pro Asp Gly Thr Ser Val Asp Val Lys Val

Thr Val Tyr Val Asp Pro Val Val Glu Ser Asn Arg Asp Thr Leu Ser 165 170 175

Lys Glu Ala Asn Thr Gly Asn Thr Asn Val Ala Lys Ala Ala Thr Val

Thr Ser Ser Lys Val Glu Ser Lys Lys Thr 195 200

<210> 7

<211> 232

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:C14 cell wall targeting region

<400> 7

Val Thr Arg Thr Ile Asn Val Val Asp Pro Ile Thr Gly Lys Ile Ser 1 5 10 15

Thr Ser Val Gln Thr Ala Lys Phe Thr Arg Glu Asp Lys Asn Ser Asn 20 25 30

Ala Gly Tyr Thr Asp Pro Val Thr Gly Lys Thr Thr Met Asn Pro Trp 35 40 45

Thr Pro Ala Lys Gln Gly Leu Arg Ala Val Asn Val Glu Gln Ile Lys 50 55 60

Gly Tyr Val Ala Lys Val Asp Gly Asn Val Asp Ala Val Val Thr 65 70 75 80

Pro Asp Ser Ala Asn Met Val Val Thr Ile Thr Tyr Gln Ala Asn Lys 85 90 95

Pro Glu Gly Gln Asn Ile Thr Val Lys Lys Asp Thr Val Pro Asp Pro 100 105 110

Ala Asp Gly Ile Lys Asn Lys Asp Asp Leu Pro Asp Gly Thr Lys Tyr 115 120 125

Thr Trp Lys Glu Val Pro Asp Val Asn Ser Val Gly Glu Lys Thr Gly 130 135 140

Ile Val Thr Val Thr Phe Pro Asp Gly Thr Ser Val Asp Val Lys Val 145 150 155 160

Thr Val Tyr Val Asp Pro Val Val Glu Ser Asn Arg Asp Thr Leu Ser 165 170 175

Lys Glu Ala Asn Thr Gly Asn Thr Asn Val Ala Lys Ala Ala Thr Val 180 185 190

Thr Ser Ser Lys Val Glu Ser Lys Lys Thr Leu Pro Gln Thr Gly Ser 195 200 205 Lys Thr Glu Gln Val Gly Ile Leu Gly Leu Ala Ile Ala Thr Val Gly 210 215 220

Ser Leu Leu Gly Leu Gly Val Asn 225 230

<210> 8

<211> 226

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:C370 cell wall targeting region

<400> 8

Lys Lys Ala Glu Glu Val Lys Asn Asn Ser Asn Ala Thr Gln Lys Glu

1 10 15

Val Asp Asp Ala Thr Asn Asn Leu Lys Gln Ala Gln Asn Asp Leu Asp 20 25 30

Gly Gln Thr Thr Asp Lys Ser Lys Leu Asp Glu Ala Ile Lys Ser Ala 35 40 45

Asp Asp Thr Lys Ser Thr Asp Lys Tyr Asn Asn Ala Ser Asp Asp Thr 50 55 60

Lys Ser Lys Phe Asp Glu Ala Leu Lys Lys Ala Glu Glu Val Lys Asn 65 70 75 80

Asn Ser Asn Ala Thr Gln Lys Glu Val Asp Asp Ala Thr Lys Asn Leu 85 90 95

Lys Gln Ala Gln Asn Asp Leu Asp Gly Gln Thr Thr Asn Lys Asp Ala
100 105 110

Ile Asn Asp Ala Ile Lys Asp Ala Asn Asn Ala Lys Gly Thr Asp Lys 115 120 125

Tyr Asn Asn Ala Ser Asp Asp Thr Lys Ser Lys Phe Asp Asp Ala Leu 130 135 140

Lys Lys Ala Glu Asp Val Lys Asn Asp Ser Asn Ala Asn Gln Lys Glu 145 150 155 160

Val Asp Asp Ala Thr Lys Asn Leu Lys Asn Thr Leu Asn Asn Leu Lys
165 170 175

Gly Gln Pro Ala Lys Lys Ala Asn Leu Ile Ala Ser Lys Asp Asn Ala 180 185 190

Lys Ile His Lys Gln Thr Leu Leu Pro Gln Thr Gly Thr Glu Thr Asn 195 200 205

Pro Leu Thr Ala Ile Gly Ile Gly Leu Met Ala Leu Gly Ala Gly Ile 210 215 220

Phe Ala

225

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<210> 9
<211> 5
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Streptococcus
      pyogenes M6 (emm6) protein signature cell wall
      sorting signal motif, substrate for sortase-like
      proteins, cell wall anchor motif
<220>
<221> MOD RES
<222> (3)
<223> Xaa = any amino acid
<400> 9
Leu Pro Xaa Thr Gly
<210> 10
<211> 6
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:C-terminus
      positive charged residues cell surface retention
      signal
<400> 10
Lys Arg Lys Glu Glu Asn
 1
<210> 11
<211> 5
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:cell wall
      targeting region
<400> 11
Leu Pro Gln Ser Gly
 1
<210> 12
<211> 5
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:cell wall
      targeting region
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<400> 12
Leu Pro Gln Ala Gly
 1
<210> 13
<211> 5
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:cell wall
      targeting region, cell wall sorting signal, cell
      wall associated (CWA) domain
<400> 13
Leu Pro Gln Thr Gly
<210> 14
<211> 5
<212> PRT
<213> Artificial Sequence
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<223> Description of Artificial Sequence:cell wall
      targeting region
<400> 14
Leu Pro Gln Thr Ala
<210> 15
<211> 10
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:c-Myc epitope,
      c-Myc tag
<400> 15
Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
                 5
                                      10
 1
<210> 16
<211> 8
<212> PRT
<213> Artificial Sequence
<220>
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      charged tail
<400> 16
Val Asn Arg Lys Lys Arg Gln Lys
  1
                  5
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<210> 17
<211> 11
<212> PRT
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      charged tail
Phe Ala Lys Lys Lys Arg Lys Asp Asp Glu Ala
<210> 18
<211> 5
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:cell wall
      sorting signal, cell wall targeting region
<400> 18
Leu Pro Gln Ser Ala
<210> 19
<211> 5
<212> PRT
<213> Artificial Sequence
<220>
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      sorting signal, cell wall targeting region
<400> 19
Leu Pro Gln Ala Ala
  1
<210> 20
<211> 19
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:C14 carboxyl
      terminus hydrophobic region spanning bacterial
      membrane
<400> 20
Val Gly Ile Leu Gly Leu Ala Ile Ala Thr Val Gly Ser Leu Leu Gly
                                                          15
                                      10
Leu Gly Val
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<210> 21
<211> 18
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:C370 carboxyl
      terminus hydrophobic region spanning bacterial
      membrane
<400> 21
Pro Leu Thr Ala Ile Gly Ile Gly Leu Met Ala Leu Gly Ala Gly Ile
Phe Ala
<210> 22
<211> 6
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:C191 exemplary
      carboxyl terminal positive charged region,
      positive charged tails
<400> 22
Lys Lys Arg Lys Glu Asp
                  5
<210> 23
<211> 6
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:C14 exemplary
      carboxyl terminal positive charged region,
      positive charged tails
<400> 23
Arg Lys Lys Arg Gln Lys
  1
<210> 24
<211> 9
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:C370 exemplary
      carboxyl terminal positive charged region,
      positive charged tails
<400> 24
Lys Lys Lys Arg Lys Asp Asp Glu Ala
                 5
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<210> 25
<211> 23
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: exemplary
      signal sequence from amino terminus of
      alpha-amylase of Lactobacillus amylovorus
<400> 25
Met Lys Lys Asn Leu Arg Ile Val Ser Ala Ala Ala Ala Leu Leu
Ala Val Ala Pro Val Ala Ala
             20
<210> 26
<211> 23
<212> PRT
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<220>
<223> Description of Artificial Sequence:exemplary
      signal sequence from amino terminus of S-layer
      gene (cbsA) of Lactobacillus crispatus
<400> 26
Met Lys Lys Asn Leu Arg Ile Val Ser Ala Ala Ala Ala Leu Leu
                                     10
Ala Val Ala Thr Val Ser Ala
             20
<210> 27
<211> 5
<212> PRT
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<220>
<223> Description of Artificial Sequence:Lactobacillus
      paracasei PrtP protease sorting signal
<400> 27
Leu Pro Lys Thr Ala
 1
<210> 28
<211> 5
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Staphylococcus
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aureus protein A cell wall sorting motif

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<400> 28
Leu Pro Glu Thr Gly
<210> 29
<211> 5
<212> PRT
<213> Artificial Sequence
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<223> Description of Artificial
      Sequence: Peptostreptococcus magnus protein L and
      human serum albumin binding protein sorting signal
      motif
<220>
<221> MOD RES
<222> (3)
<223> Xaa = any amino acid
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Leu Pro Xaa Ala Gly
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<210> 30
<211> 5
<212> PRT
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      paracasei sorting signal motif
<220>
<221> MOD RES
<222> (3)
<223> Xaa = any amino acid
<400> 30
Leu Pro Xaa Thr Ala
 1
<210> 31
<211> 6
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Gram-positive
      bacterial cell wall anchored protein conserved
      C-terminal motif
<220>
<221> MOD_RES
<222> (1)..(6)
<223> Xaa = any amino acid
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<400> 31
Leu Pro Xaa Thr Gly Xaa
                  5
<210> 32
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<212> DNA
<213> Artificial Sequence
<220>
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gtggagctcc ccgaaaagcc ctgacaaccc
                                                                    30
<210> 33
<211> 26
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ggaaacacgc tagcactaac ttcatt
<210> 34
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<400> 34
gcggctagca agaaagttgt tttaggtaaa
                                                                    30
<210> 35
<211> 30
<212> DNA
<213> Artificial Sequence
<220>
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      amplification oligonucleotide primer 2DCD4.r
<400> 35
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gcacaattgt gatgcctttt gaaaagctaa
<210> 36
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<212> DNA
<213> Artificial Sequence
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<220>
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      amplification oligonucleotide primer CbsAss.f
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                                                                    27
gcgaattcaa ggaggaaaag accacat
<210> 37
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<212> DNA
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<400> 37
                                                                    27
ccagctagct gaaacagtag aaacggc
<210> 38
<211> 59
<212> DNA
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<220>
<223> Description of Artificial Sequence:5'
      oligonucleotide primer Myc14nhe
<400> 38
gcgctagcga acagaaactg atctccgaag aggacctggt aactcgtact atcaatgta 59
<210> 39
<211> 30
<212> DNA
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<220>
<223> Description of Artificial Sequence:3'
      oligonucleotide primer Myc14mfe
<400> 39
cgccaattgc tacttttgac gtttctttct
                                                                    30
<210> 40
<211> 57
<212> DNA
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      oligonucleotide primer Myc191nhe
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<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence
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                                                                    30
<210> 42
<211> 58
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      oligonucleotide primer Myc370nhe
<400> 42
gcgctagcga acagaaactg atctccgaag aggacctgtt gaagaaggca gaagaagt
                                                                    58
<210> 43
<211> 30
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      oligonucleotide primer Myc370mfe
<400> 43
ccgcaattgt tatgcttcat catcttttct
                                                                    30
<210> 44
<211> 30
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:C14 PCR
      amplification 5' primer
<400> 44
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gcgcaattgg taactcgtac tatcaatgta
<210> 45
<211> 30
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<220>
<223> Description of Artificial Sequence:C14 PCR
      amplification 3' primer
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<210 > 46 <211 > 30 <212 > Di <213 > Ai	0	
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<400> 46 gcgcaatt		30
<210> 47 <211> 30 <212> Di <213> An	0	
	escription of Artificial Sequence:C191 PCR mplification 3' primer	
<400> 47		3 0
	6	
	escription of Artificial Sequence:C370 PCR mplification 5' primer	
<400> 48 gcgcaatt		26
<210> 49 <211> 30 <212> DN <213> An	0	
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<400> 49 ccgtctag		30
<210> 50 <211> 30 <212> DI <213> A	0	

<220> <223> Description of Artificial Sequence:shuttle vector cloning primer Mfec14up	
<400> 50 gcgcaattgc cacaaactgg ttctaagact	30
<210> 51 <211> 30 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence:shuttle vector cloning 3' primer Xnac14lo	
<400> 51 cgctctagat acacaaacta ttttacggtc	30
<210> 52 <211> 26 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence:C370 repetitive cell wall spanning region zero repeat PCR amplification 5' primer	
<400> 52 cggcaattgc ctcaaactgg tactga	26
<210> 53 <211> 30 <212> DNA <213> Artificial Sequence	
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<400> 53 cggcaattgg gtcaaactac aaataaagat	30
<210> 54 <211> 30 <212> DNA <213> Artificial Sequence	
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<210> 55 <211> 30 <212> DNA <213> Artificial Sequence	
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<400> 55 gcgcaattgg gtcaaactac aaataaagat	30
<210> 56 <211> 30 <212> DNA <213> Artificial Sequence	
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<400> 56 cggcaattgg gtcaaactac tgacaagagc	30
<210> 57 <211> 42 <212> DNA <213> Artificial Sequence	
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<400> 57 gaaagtaaga agactttacc acaaactggt tctaagactg aa	42
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<400> 58 cataagcaaa ctctattgcc tcaaactggt actgaaacta acccac	46
<210> 59 <211> 37	
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<223>	Description of Artificial Sequence:C14 site-directed mutagenic primer 237P(A)	
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<210>		
<211><212>	·	
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<220>	·	
	Description of Artificial Sequence:C14 site-directed mutagenic primer 237P(A)	
<400>		
gtctta	gaac cagtttgtgc taaagtcttc ttactttc	38
<210>	61	
<211>	·	
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	ATTITICIAL DEQUENCE	
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(223)	site-directed mutagenic primer 237P(N)	
<400>	61	
gaaagt	aaga agactttaaa tcaaactggt tctaagac	38
<210>	62	
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<400>	62	
gtctta	gaac cagtttgatt taaagtcttc ttactttc	38
-210-		
<210><211>		
<212>		
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<220>		
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agaaga	cttt accacaagct ggttctaaga ctgaac	36

-210-	64	
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-213-	Artificial Sequence	
\Z1J/	Artificial bequence	
<220>		
<223×	Description of Artificial Sequence:C14	
12237		
	site-directed mutagenic primer 237T(A)	
<400>	64	
attead	stott agaaccagot tgtggtaaag tottot	36
50000	agaaccagee cycyyeanag cocce	
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<211>	36	
<212>		
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<223>		
	site-directed mutagenic primer 237T(G)	
<400>	65	
		36
agaaga	acttt accacaaggt ggttctaaga ctgaac	20
<210>	66	
<211>		
<212>	DNA	
<213>	Artificial Sequence	
222		
<220>		
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	site-directed mutagenic primer 237T(G)	
	into allocota into agoing primer to the (e)	
<400>	66	
gttcad	tett agaaccacet tgtggtaaag tettet	36
-		
<210>	67	
<211>	36	
<212>	DNA	
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\2237		
	site-directed mutagenic primer 237T(S)	
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agaaga	acttt accacaaagt ggttctaaga ctgaac	36
agaaga	dett detacade ggetteadga tegade	_
<210>	68	
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<212>		
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<210> 71 <211> 38 <212> DNA <213> Artificial Sequence	
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<400> 71 cataagcaaa ctctattggc tcaaactggt actgaaac	38
<210> 72 <211> 38 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence:C370 site-directed mutagenic primer 249P(A)	
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<210> 73 <211> 38 <212> DNA <213> Artificial Sequence	

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<400> gtttca	74 agtac cagtttgatt caatagagtt tgcttatg	38
<210><211><212><212><213>	35	
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<210><211><211><212><213>	36	
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<210><211><211><212><213>	36	
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<220> <223>	Description of Artificial Sequence:C370 site-directed mutagenic primer 249T(S)	
<400> gttagt	· · · · · · · · · · · · · · · · · · ·	36
<210><211><211><212><213>	36	
<220> <223>	Description of Artificial Sequence:C370 site-directed mutagenic primer 249G(A)	
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<223>	Description of Artificial Sequence:C14-7 reverse amplification primer oligonucleotide	
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<210><211><212><212><213>	35	
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<400> gcgctc		36
<210><211><212><213>	34	
<220> <223>	Description of Artificial Sequence:370-8 reverse amplification primer oligonucleotide	
<400> gcgctc		34